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WITNESS my hand this Fifteenth day of October 1998

KIM MARSHALL
MANAGER EXAMINATION SUPPORT AND SALES

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CONP 10/11

AUSTRALIA

Patents Act 1990

Macquarie Research Ltd

PROVISIONAL SPECIFICATION

Invention Title:

Sampling device

The invention is described in the following statement:

Technical Field

The present invention relates to a sampling device for removing one or more samples from an array of samples. Typically, the samples are biomolecules in a gel or solid support.

Background Art

Improvements in laboratory techniques and practices have led to the discovery of an ever increasing number of new biomolecules. New protein purification and detection methods, for example, have allowed the detection of many possibly new proteins. Due to the large number of known biomolecules, it is now necessary to carry out molecular comparisons of newly discovered molecules to determine to what extent they are similar to or different from know molecules. For example, to carry out definitive analysis for proteins it is necessary to obtain amino acid sequence information or determine the masses of peptides after protein digestion. Generally, the biomolecules for analysis are present as "concentrated" spots on either dry polymer membranes or wet gels. In order to carry out analysis of the biomolecules the spots are cut out by a laboratory worker or researcher using a scalpel and are placed in a test tube for analysis by application of a reagent or succession of reagents. Generally only one sample can be analysed at a time. Typically a single membrane may have many hundreds or thousands of spots of biomolecules and clearly the process is extremely laborious.

The present invention seeks to overcome or at least ameliorate some of the problems associated with the background art discussed above.

Disclosure of the Invention

According to the present invention there is provided a sampling device comprising:

a) a table means for supporting an array of samples;

b) means for recording an electronic image of at least a part of the array of samples;

c) display means for displaying the electronic image of the array on a screen or the like;

d) sampling means operable to sample one sample from the array of samples, pick up the one sample, and release the one sample;

e) means for selecting a sample on the array for sampling by the sampling means;

f) means for moving the table means relative to the sampling means in the plane of the array; and

g) control means operatively connected to the sampling means for controlling the operation of the sampling means and movement of the same relative to the table means.

Typically the array of samples will be present as a non-ordered array of spots on dry polymer membranes or wet gels.

The sample will preferably be of a biological nature and may include proteins, peptides, polysaccharides, lipids and nucleic acid molecules or complex molecules like glycoproteins, for example.

The means for recording an electronic image of at least a part of the array of samples may be a digital camera. The electronic image may be generated from a scan of the samples stained or illuminated or otherwise marked with a visible or fluorescent marker to allow them to be visualised. An ink jet dispensing unit, such as is disclosed in applicants co-pending Australian patent application No PO6254, the entire contents of which are incorporated herein by reference, could be used for marking the spots/samples.

In a preferred embodiment an image file relating to each array of samples is stored on a computer. An image of the array is displayed on the computer and spots are selected from the array to be sampled using the computer monitor and a mouse. Once a mouse is clicked on a particular spot the sampling device will automatically move to that spot, cut the spot from the array, pick up the spot and transport it to an appropriate receptacle such as a test tube, ninety-six well plate, or the like.

In a preferred embodiment the array of samples is in a plane, the x-y plane, and the table means is movable in both the x and y directions so that the spot to be sampled is placed underneath the sampling means.

Alternatively, it would be possible to have the table means fixed and the sampling means movable.

In a preferred embodiment the sampling means is a cutting device which is adapted to cut a spot/sample from out of the array of samples.

However it would be possible for the sampling means to merely remove a portion of the spot.

In a related aspect the invention provides a cutting head comprising:

punch means defining a central bore adapted to cut and retain a spot of material;

a plunger made of ferro magnetic material disposed in the bore defining a rod which is disposed in and movable along the bore;

a solenoid disposed around the plunger, wherein operation of the solenoid causes the plunger to move to eject the spot.

Preferably the cutting device is pneumatically operated. The punch may be circular.

The invention will now be described by way of example only and with reference to the accompanying drawings in which:

Figure 1 is a front view of a sampling device embodying the present invention;

Figure 2 is an enlarged front view of a cutting tool assembly which forms part of the sampling device of Figure 1;

Figure 3 is a side view of the cutting tool assembly shown in Figure 2;

Figure 4 is a plan view of the sampling device shown in Figure 1, in particular illustrating the movement of the table on an x-y grid;

Figure 5A and 5B are detailed views of the cutting head of the cutting tool assembly, where 5A shows a sample pick-up and 5B a sample eject; and

Figures 6a to 6h show the sequence of operation of the cutting tool assembly showing in sequence the cutting of a sample from an array of samples and the placing of that cut sample in a test tube.

Detailed Description of the Preferred Embodiment

Referring to the drawings, Figure 1 shows a sampling device embodying the present invention generally indicated at 10. The sampling device includes a table 12 and a overhead beam 14 which is spaced and supported above the table by two columns 16 and 18. In the centre of the beam a cutting tool assembly 20 is located which is described in more detail below. The table 12 is mounted so as to be moveable in the x and y directions in a generally horizontal plane, see Figure 4. By moving the table in the x and y directions any part of the table may be located under the cutting tool assembly 20. Motors and control means, not illustrated, are provided for moving the table. The specific means for moving the table in the x and y directions do not form part of the present invention.

Figure 2 and Figure 3 show an enlarged front view and side view respectively of the cutting tool assembly. The cutting tool assembly 20 is supported on a guide means 22 which maintains the components of the assembly in their correct orientation. At the upper end of the cutting tool assembly there is a top cylinder plate 24. Spaced below the top cylinder plate there is a bottom cylinder plate 26. A cylinder 28 is disposed between the two plates. The cylinder forms part of a pneumatic piston and cylinder arrangement which includes a piston rod 30 disposed in the cylinder 28. The free end 32 of the piston rod is fixed to a nylon block which defines a punch carriage 34 disposed directly below the bottom cylinder plate 26. Movement of the piston rod 30 in the rod cylinder 28 in the vertical (z axis) direction causes the punch carriage 34 also to move in the vertical direction guided by guide rods. Movement of the piston in the cylinder is pneumatically controlled.

The punch carriage 34 defines a cylindrical aperture or bore 35 which extends up from the lower face 34A of the punch carriage, towards the piston rod 30 and the upper end 34B of the punch carriage. A cutting head assembly generally indicated at 36 is disposed inside the aperture and is shown in more detail in Figures 5A and 5B.

Turning now to Figures 5A and 5B, a solenoid 38 is disposed in the interior of the aperture 35. A generally cylindrical nylon block 40 is fixed in the centre of the aperture 35 and is directly in line with the piston rod 30. A punch holder 42 is disposed inside a central bore of the block 40. The punch holder defines a central bore 44 in which a steel plunger 46 is located. The plunger 46 is generally cylindrical and defines a large diameter body portion 48 from one lower end 48A of which there projects a narrower cylindrical rod 50. A spring 52 is disposed between the other opposite end 48B of the plunger and the upper end of the central bore 44.

A punch 54 is fixed to the lower end of the punch holder/cutting head assembly. A generally cylindrical bore 56 extends through the centre of the punch 54 in which the cylindrical rod 50 of the plunger is disposed. The rod is coated with acetyl a low friction material such as Teflon™ or ACETYL™ both as a barrier to prevent residue from previously picked up samples from contaminating subsequently taken samples and to reduce friction between the rod and bore 56. The lower end of the punch defines a generally circular cutting blade 58, similar to a cookie cutter. As can be seen

from a comparison of Figures 5A and 5B, when the cutting head is in a "pick up" position the end of the rod 50 is withdrawn into the plunger and the end of the plunger defines a small volume which can receive a portion of material cut from the array by the cutting blade 58 of the plunger.

In Figure 5B shows when the rod 46 is moved sufficiently towards the punch 54 the distal or lower end 50A of the rod/plunger extends beyond the end of the punch 54 thus ejecting any material located in the end of the punch.

Figures 6a to 6h show the cutting and transfer sequence of the sampling device of the present invention in sequence in more detail. As shown in Figure 6a initially the cutting head is disposed above a particular spot which is to be cut out of for example a solid (i.e. non-gel) support which may be an immobilisation membrane. The punch carriage 34 is located at the upper end of its travel and the end 50A of the plunger projects beyond the end of the punch 54.

Once the cutting head has been positioned above the correct spot, the punch carriage is operated by the pneumatic piston and cylinder assembly to push the cutting head downwards until the end 50A of the plunger touches the spot/sample to be cut. This contact secures the spot and ensures it does not move. Further pressure exerted by the pneumatic cylinder will, as shown in Figure 6c, cause the punch to move relative to the plunger and cut the spot away from the surrounding membrane, while compressing the spring 52. The plunger retracts and retains the cut sample in the manner of a vacuum pick up.

Once a spot has been collected in the cutting head assembly with the plunger retracted, as seen in Figure 6e, the table is then moved by the control means to position a test tube 102 below the cutting head, see Figure 6f.

Once the test tube 102 or a sample bottle or the like is positioned below the cutting head, the cutting head is moved downwards towards the test tube so that the plunger is positioned inside the test tube.

The plunger is then pushed downwards by activating the solenoid which causes the plunger to move downwards and eject the sample 104.

Once the sample has been ejected into the correct test tube the cutting head will then automatically move to cut the next sample.

The procedure is slightly different when cutting a spot from a sheet of gel to allow for the fact that gel is easily squashed. In the first stage of the procedure, when the punch head is lowered to contact the gel, the plunger is retracted. This prevents squashing of the gel which would occur if the end 50A projected below the punch and were forced onto the gel. The procedure for ejecting the gel spot is different also. After the cutting head has been positioned in the test tube lifting of the head is commenced fractionally (say 1ms) before the solenoid is activated to eject the gel spot, also to prevent squashing the spot.

The device also includes a digital camera adapted to create an image of the gel or membrane on which the spots are located and store them in a computer. The computer is programmed with software which takes account of the distortions produced by the digital camera when imaging the array and produces a sufficiently accurate distortion free map of the array which accords with the mechanical frame used to control the cutting head assembly. There is also no need for the gel or membrane to be exactly planar. Different programs are provided for gel and non-gel arrays to account for the slightly different procedures described above.

When it is desired to select a spot, the image of the array can be displayed on a computer monitor and a mouse moved by an operator to identify the correct spot to be sampled by the sampling device.

Thus the present invention allows a vary laborious job of a researcher individually cutting spots from a gel or support using a scalpel and allows the operation to be carried out automatically.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Dated this twenty-fifth day of September 1997

MACQUARIE RESEARCH LTD
Patent Attorneys for the Applicant:

F.B. RICE & CO.

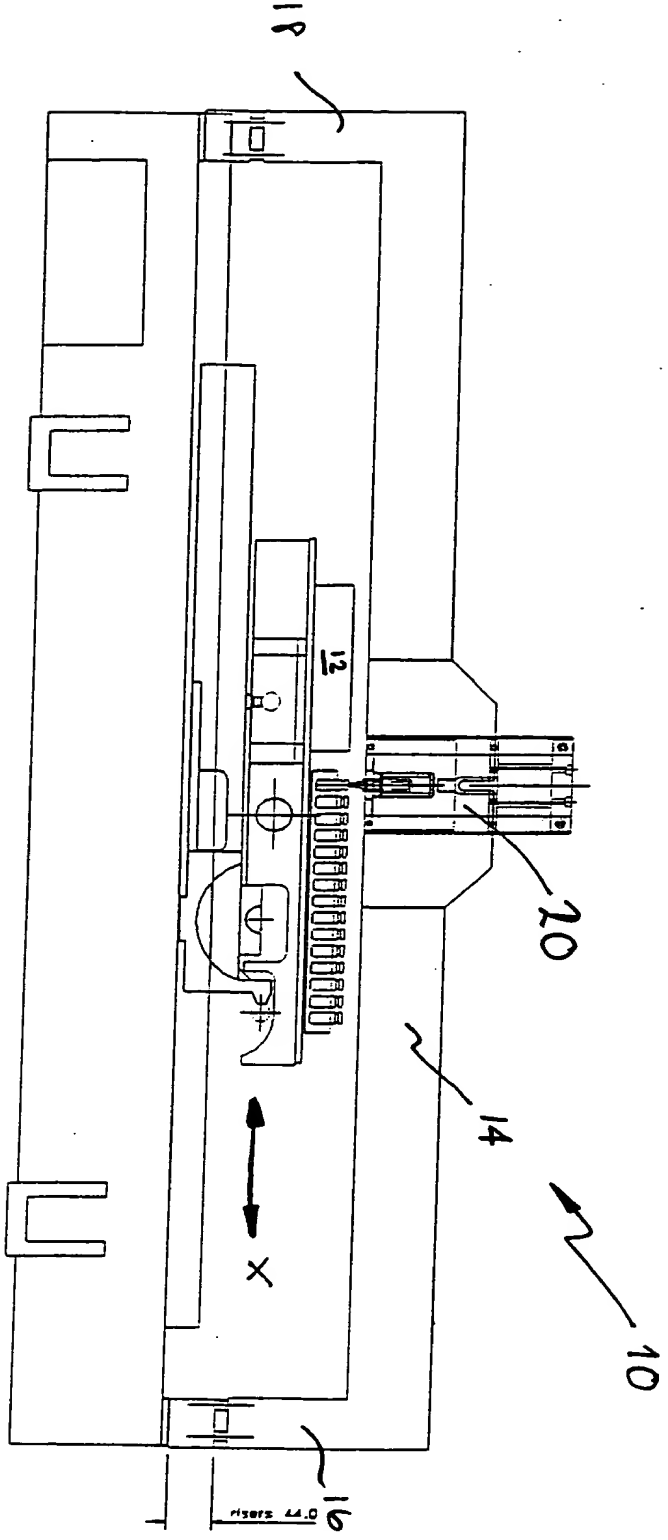


FIG. 1

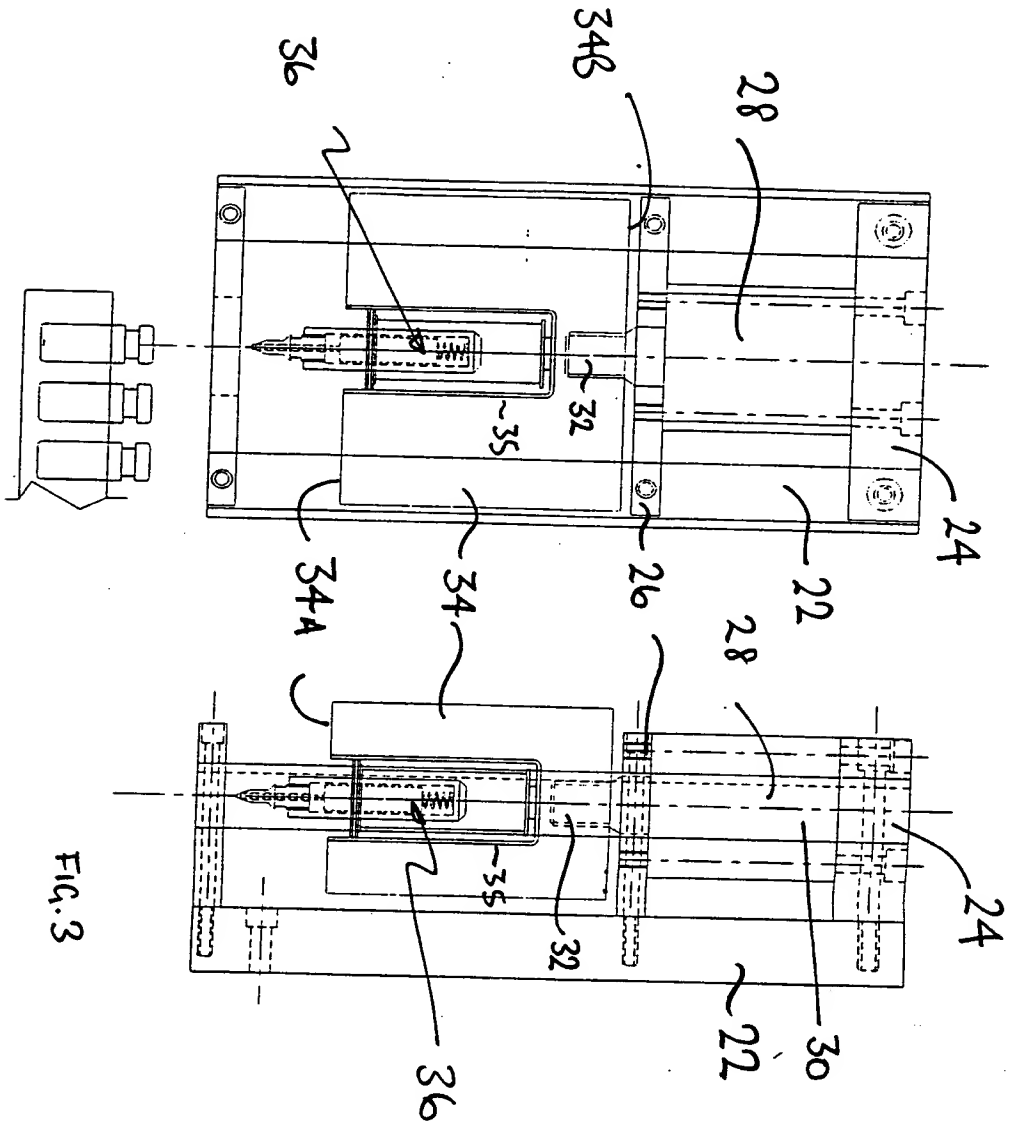


FIG. 2

FIG. 3

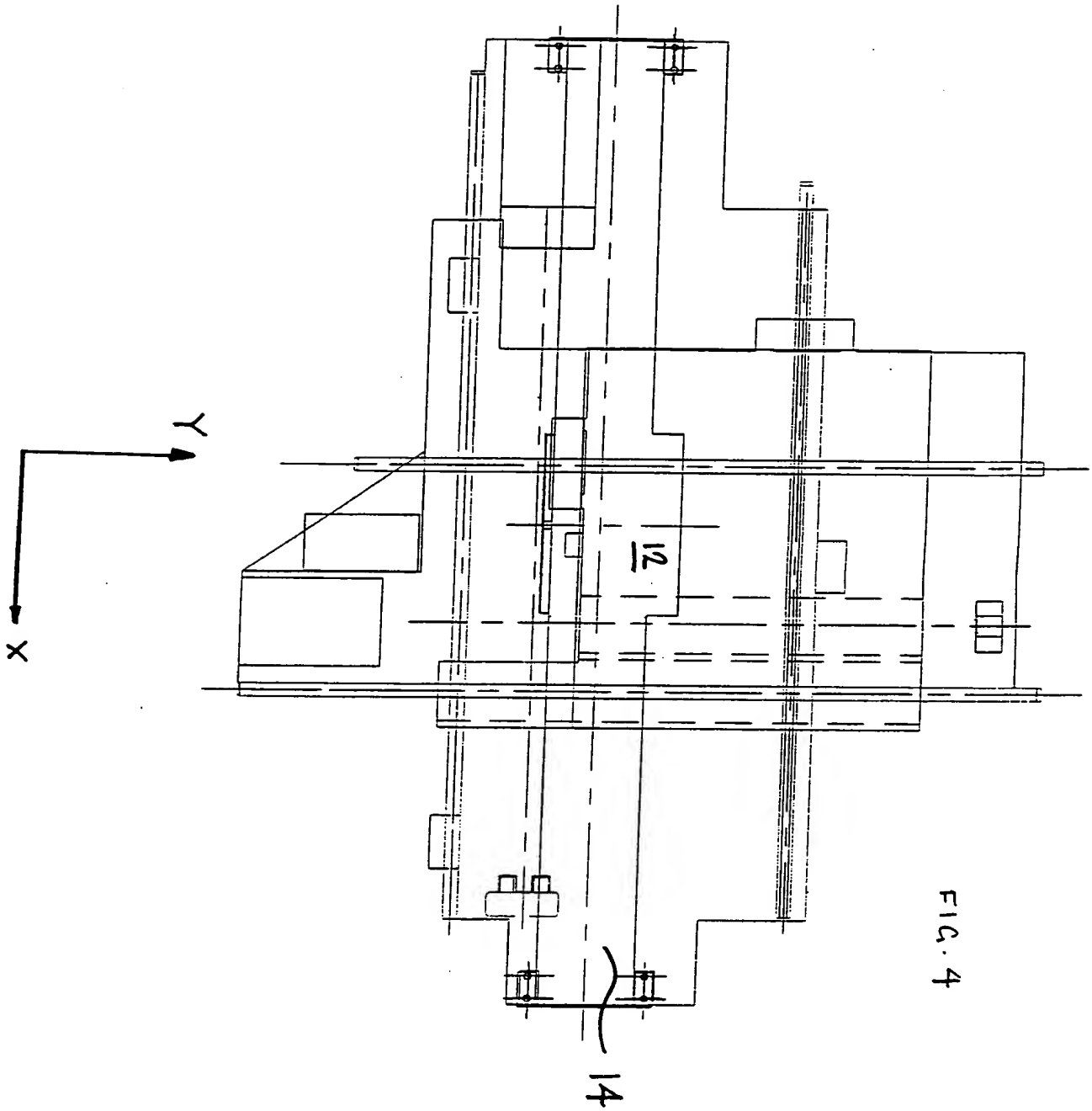
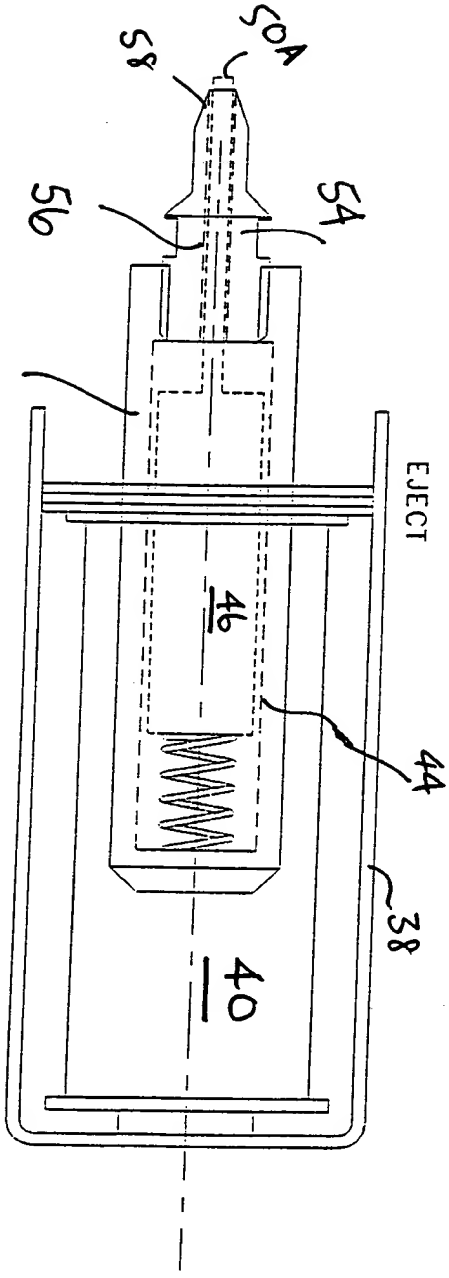


FIG. 4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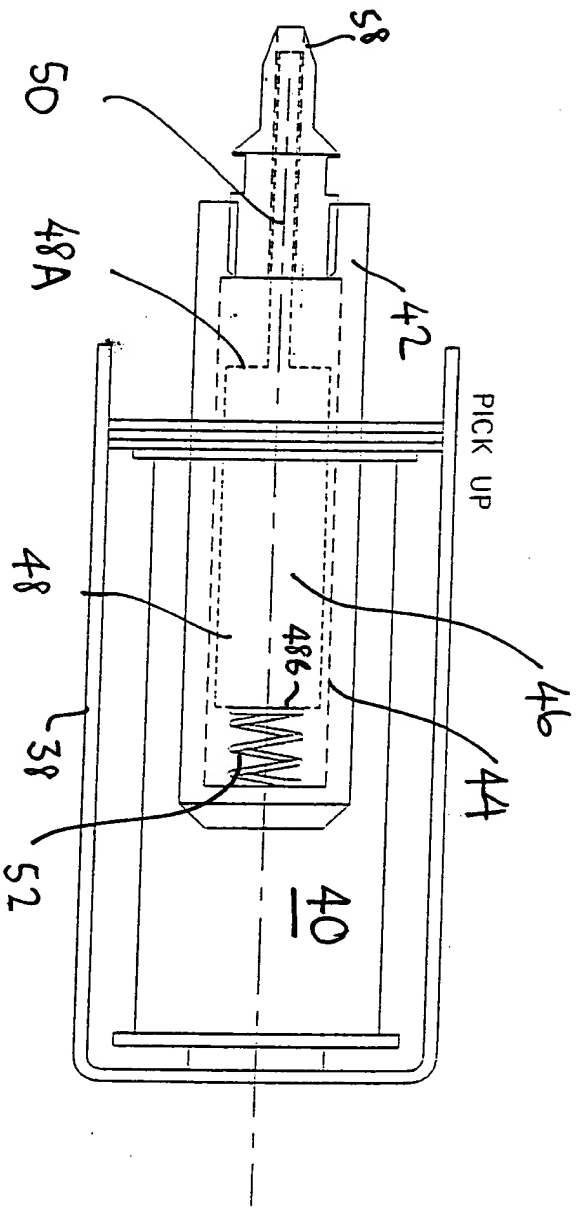
50A
56
54
42
EJECT
46
44
38
40

FIG. 5B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50
48A
42
PICK UP
48
46
44
38
52
40

FIG. 5A

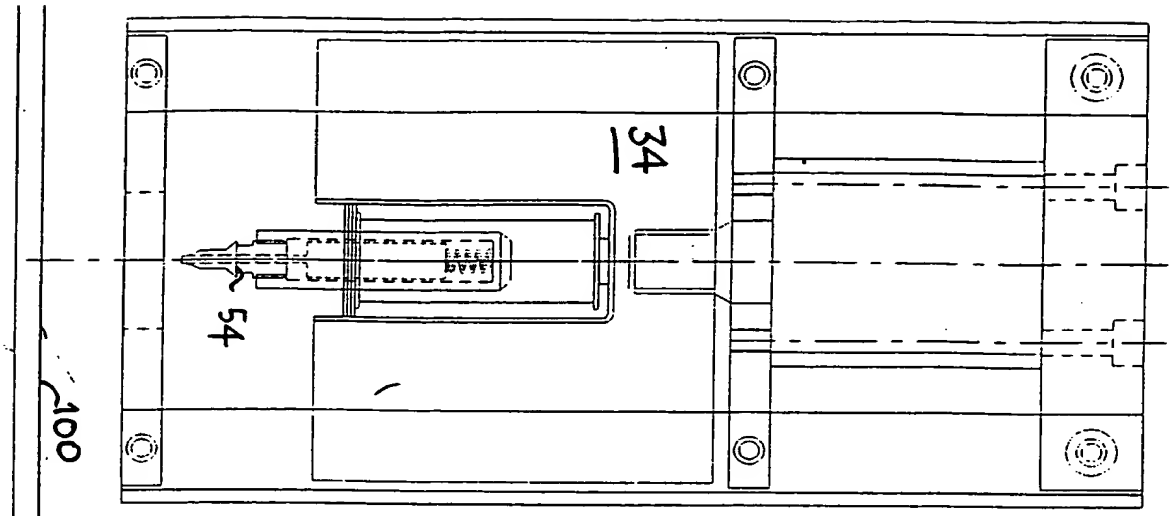


Fig. 6a

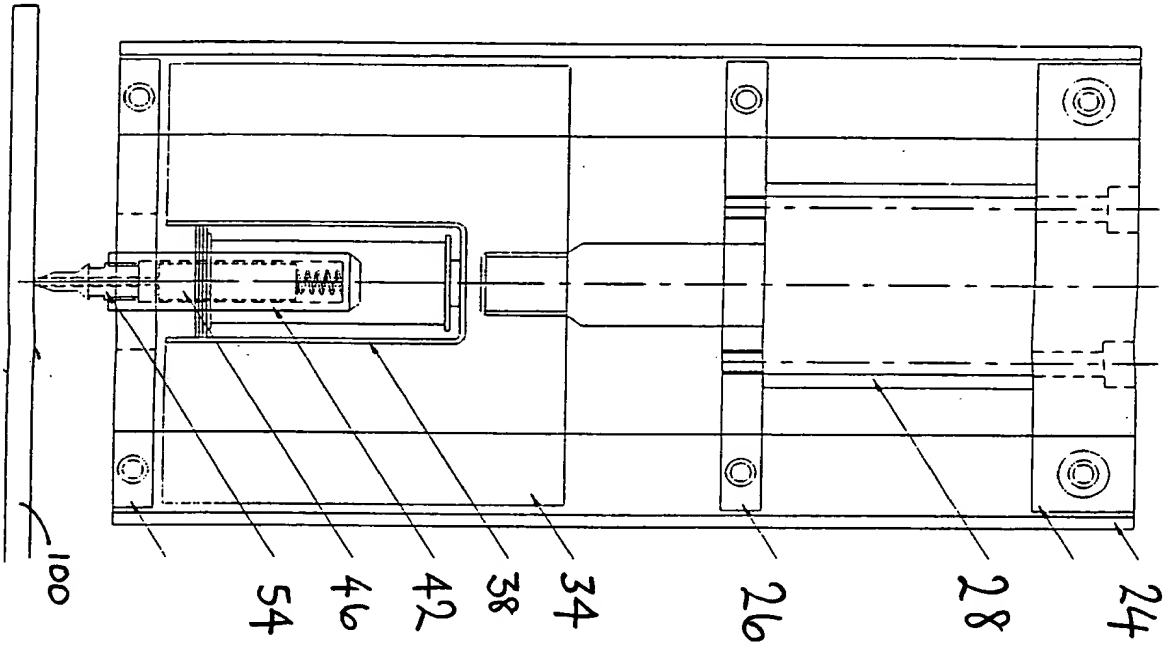


Fig 6b

Fig. 6c

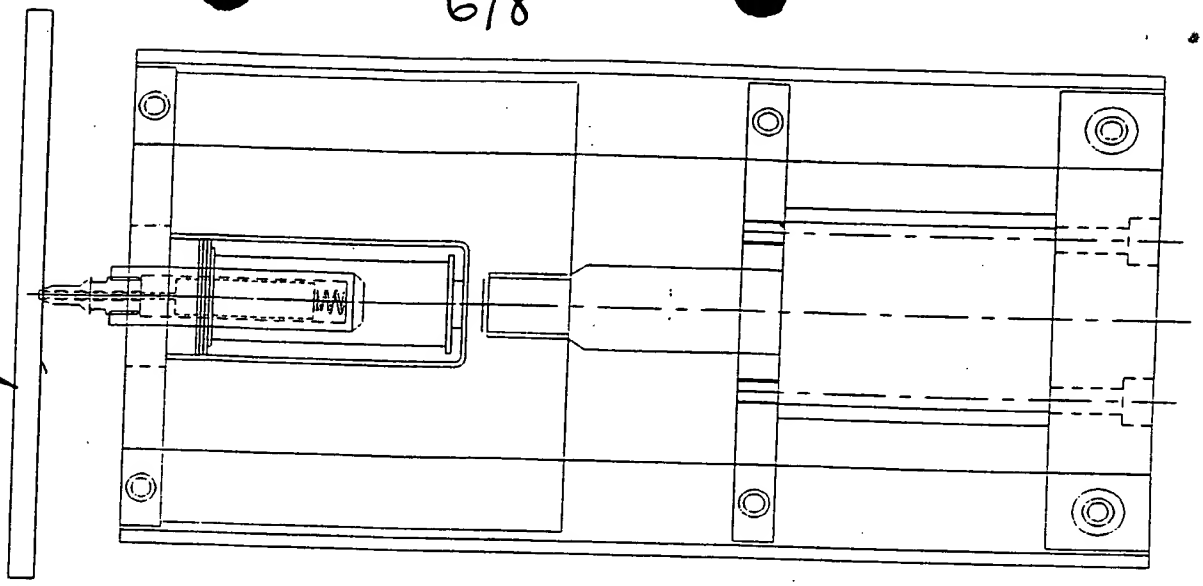


Fig. 6d

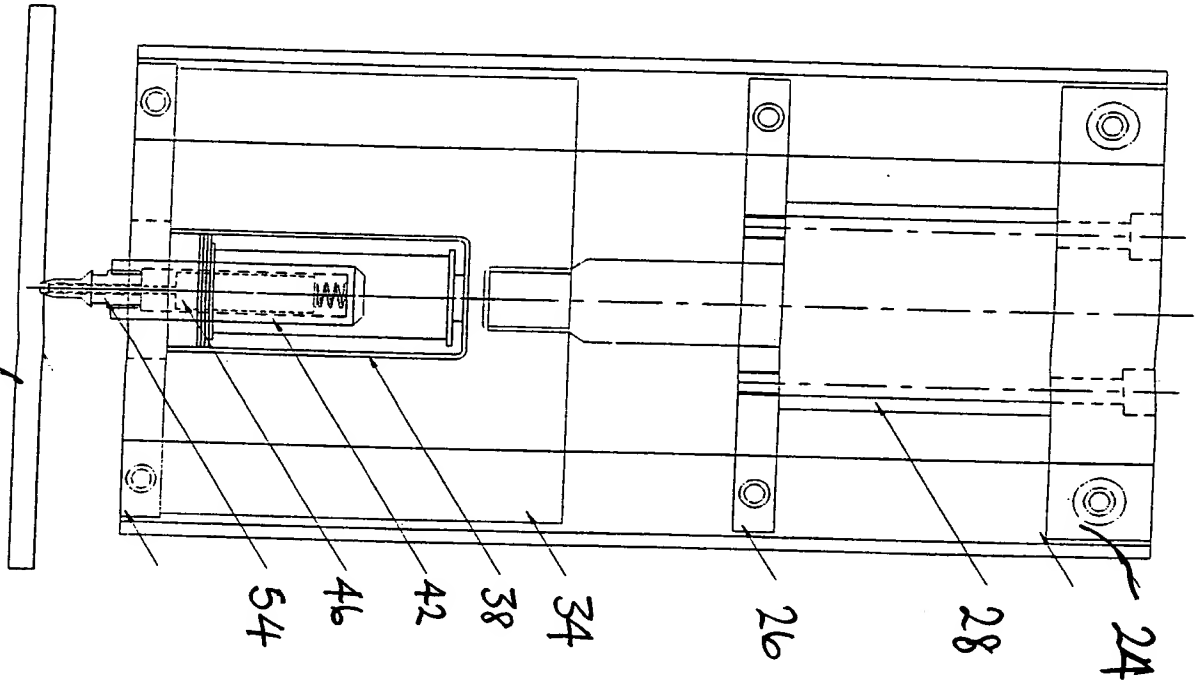


FIG. 6e

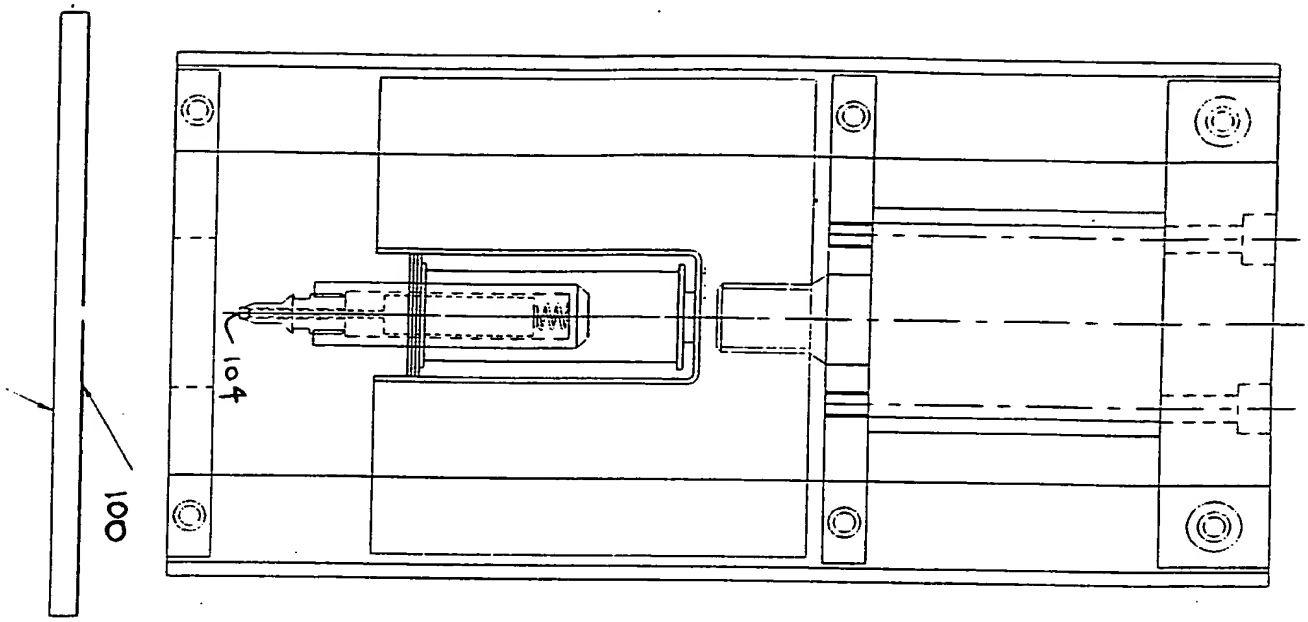


FIG. 6f

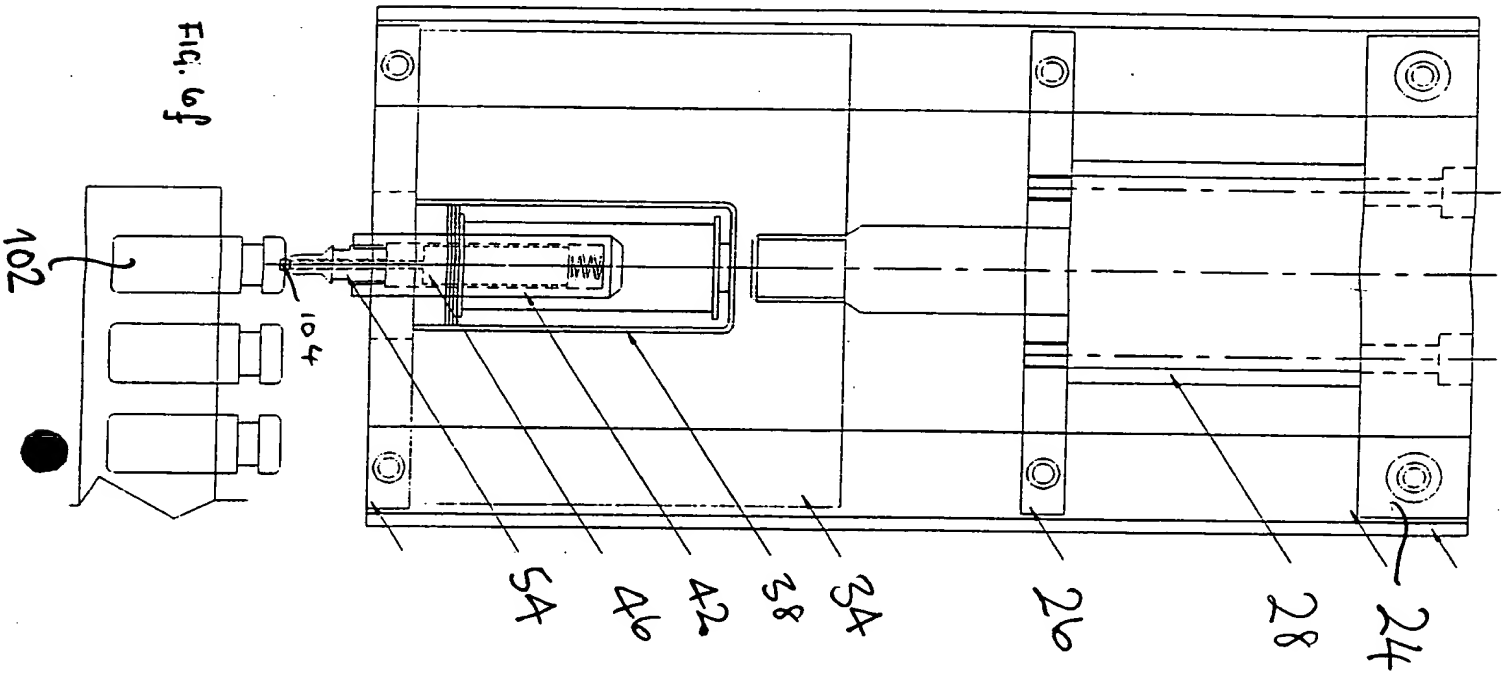


Fig. 6g

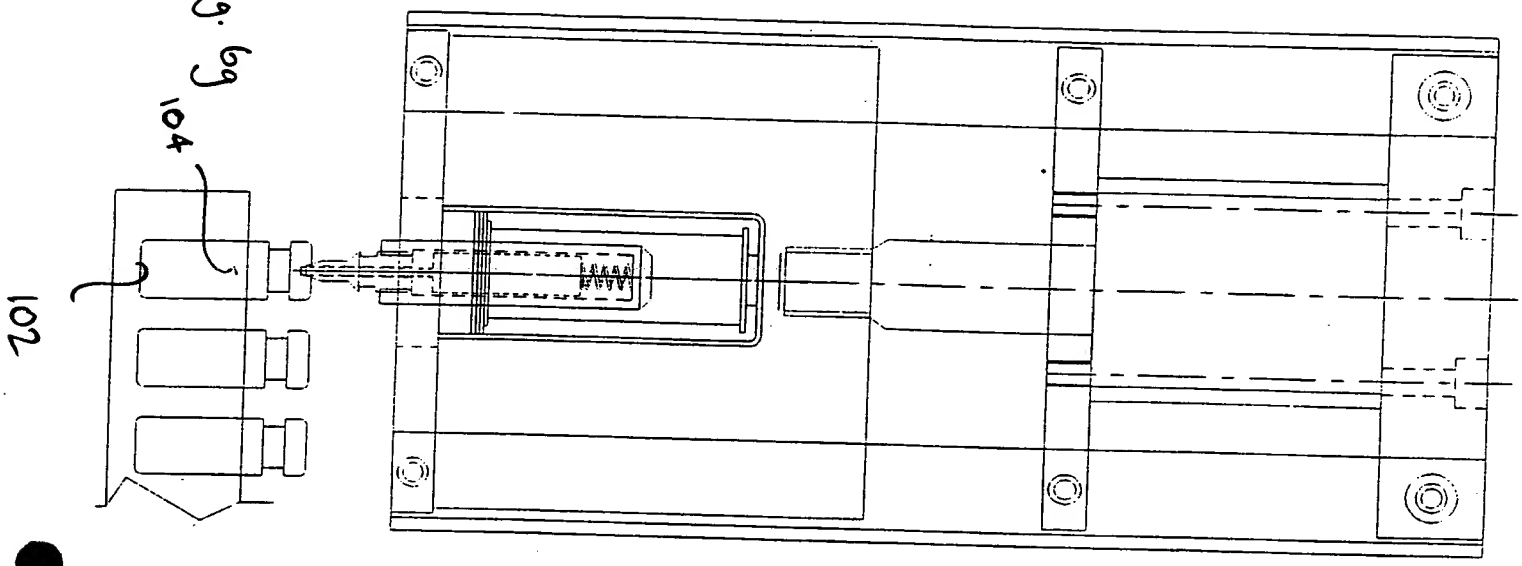
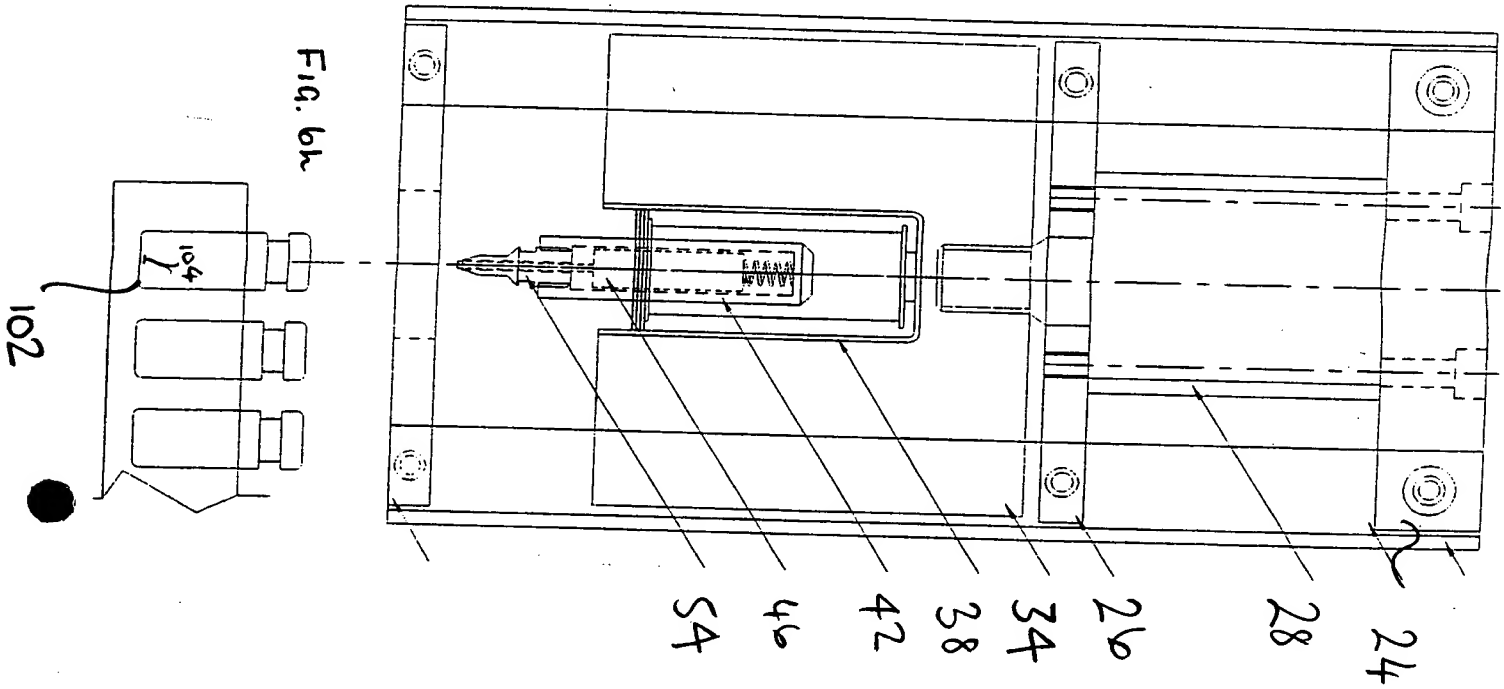


Fig. 6h



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WITNESS my hand this Fifteenth day of October 1998

KIM MARSHALL
MANAGER EXAMINATION SUPPORT AND SALES

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AUSTRALIA

Patents Act 1990

MACQUARIE RESEARCH LTD

PROVISIONAL SPECIFICATION

Invention Title:

A cutting device for removing a sample from an array of samples

The invention is described in the following statement:

Technical Field

The present invention relates to a cutting device for removing one or more samples from an array of samples. In particular, the invention relates to an apparatus for excising and ejecting biomolecules from an array of biomolecule samples in a gel or solid support.

Background Art

Multiple sample handling in laboratory techniques can be labor intensive, prone to error and contamination. At the same time, improvements in laboratory techniques and practices have led to the discovery of an ever increasing number of new biomolecules. New protein purification and detection methods, for example, have allowed the detection of many, possibly new, proteins. Due to the large number of known biomolecules, it is now necessary to carry out molecular comparisons of newly discovered molecules to determine to what extent they are similar to, or different from, known molecules. For example, to carry out definitive analysis for proteins, it is necessary to obtain amino acid sequence information or determine the masses of peptides after protein digestion. Often, the biomolecules are separated by electrophoresis in polymer based media. It is necessary to excise the biomolecule from the media and transfer them separately to a vessel such as a microtitre plate.

The present inventors have now realised that it is possible to develop an improved apparatus suitable for the excision and placement of biomolecules within polymer-based media into a vessel such as a microtitre plate.

Disclosure of Invention

In a first broad aspect, the present invention relates to a method for excising at least one sample in an array of samples comprising:

(a) recording an electronic image of the position of at least one sample relative to the other samples in the array;

(b) utilising the recorded image to control a cutting tool to excise the at least one sample; and

(c) ejecting the at least one excised sample.

In a related broad aspect, the present invention relates to an apparatus for excising at least one sample from an array of samples comprising:

(a) means for recording an electronic image of the position of at least one sample relative to the other samples in the array;

(b) means for utilising the recorded electronic image to control excising means to enable the excision of the at least one sample from the array;

(c) means for ejecting the at least one excised sample from the excising means; and

(d) control means for controlling means (a), (b), and (c), the arrangement being such that means (b) excises the sample of the at least one sample according to the position of the sample relative to the other samples in the array as determined by means (a).

Preferably, the array of samples is in a plane in order to assist in the recording of the electronic image.

The steps (a) to (c) may be repeated or cycled so as to carry out a series of excisions of a number of different samples in the array.

In a preferred embodiment of the present invention, the electronic image is generated from a digital photograph of the samples which are stained or illuminated to make the samples to be visible, and their coordinates are recorded. These coordinates are then transformed into robotic language and used to control a cutting tool whereupon the cutting tool can be directed to a selected sample.

In a particular embodiment of the present invention the cutting tool comprises:

(a) a cutting tip means having a bore therethrough;

(b) a cutting tip holder for holding the cutting tip means;

(c) an ejector pin one end of which is disposed in bore of the cutting tip, the pin being moveable along the bore of the cutting tip;

(d) a magnet or a piece of ferromagnetic material attached to the ejector pin distal from the one end;

(e) a solenoid disposed around the magnet or ferro magnetic material for causing the pin to move in the bore in a direction which expels material from the cutting tip when the solenoid is energised; and

return means for causing the pin to move in the opposite direction when the solenoid is not energised.

If item (d) is a magnet the return means may also be a magnet. The return means may also be a spring.

Preferably the cutting tip is removable and disposable.

The sample is typically of a biological nature and may include proteins, peptides, polysaccharides, lipids, and nucleic acid molecules or complex molecules like glycoproteins, for example.

The samples may be separated by known means such as electrophoresis in a polymer matrix which is then placed on a solid support or membrane support. For example, two dimensional electrophoresis separations in polyacrylamide are transferred to supports like PTFE, gortex, PVDF, nylon, nitrocellulose, polypropylene which are particularly suitable for supporting an array of samples for excision using the methods and apparatus of the present invention. Samples may also be excised without having to transfer them to a membrane.

In order to generate an electronic image of the samples in the array, it is necessary to make them identifiable in some manner. Labelling the samples with a visible marker is one example that would allow the visualisation of the position of the samples with a photodetector. A scan of the labelled samples would then be recorded electronically and stored in a computer, for example. Once the electronic image has been recorded there would be no need to maintain the visualisation of the samples on the array as the image is maintained electronically. If the location of the samples are recorded on an X/Y grid, this would be one way of accessing the positions of the samples electronically. The computer would also control the cutting tool in step (b) to the position of the sample to be excised. The position of all the samples would be known from their co-ordinates on the grid, and so excision is possible regardless of whether or not the samples are still visible. Once excised the cutting tool moves to the defined coordinate of a well of a microtitre plate and the sample is ejected.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

In order that the present invention may be more clearly understood, preferred forms will be described with reference to the following examples and accompanying drawings.

Brief Description of Drawings

Figure 1 shows a schematic of the electronic recording and sample excision robot aspects of an apparatus according to the present invention;

Figure 2a shows a first embodiment of an excision tool; and

Figure 2b shows a second embodiment of an excision tool.

Detailed Description Of The Presently Preferred Embodiments

Turning now to the drawings, FIG. 1 shows a schematic representation of a first preferred embodiment of a robotic excision apparatus. The robotic excision apparatus 100 includes an image acquisition system 200 which includes a camera, an excision tool 400, and a computer 300. An array of samples is placed onto a silicon mat 105 which is housed inside an acrylic base plate 101 which is illuminated from underneath the sample with fluorescent light (for acrylamide) or from above with tungsten lamps or a camera flash (for membranes) 106. The image is transferred from the image acquisition means to the computer 300. The image is processed and imported into "click-on-a-spot" software. This process translates the image pixel coordinates into robot coordinates. The "click-on-a-spot" software is then used to drive the excision tool 400 to the selected component via an x, y movable bar 102. The z movement of the excision tool 400 is via an excision tool support unit 107. The excision tool 400, which is described in more detail below, then cuts out and holds the selected sample and moves above a specific well of a microtitre plate into which the sample is to be placed. The excised sample is then deposited into the specific well of the microtitre plate 108.

A first embodiment of the excision/cutting tool is shown in FIG 2a.

The cutting tool comprises a cylindrical body portion 400, which has an upper end 400A and a lower end 400B. The body portion is a generally cylindrical tube defining a central bore 408 and can be made of metal or hard plastic or any suitable material.

The lower end 400B of the tube is closed with a end portion which acts as a tip holder 404 which has a central cylindrical bore in which is mounted a tip 409. The tip 409 has an annular cross section and has a wider cylindrical portion which locates inside the bore 408 of the body and after it

emerges below the tip holder then tapers generally conically to a narrower portion which acts as a cutting head 406. The tip, and tip holder are fixed relative to the body portion.

The tip can be made of various materials including glass, metal or plastic, however, it is preferable if the tip is translucent as this makes it possible to determine if acrylamide residue is caught inside the tip. The tip may be removable.

The pin is not illustrated in Figure 2a but its upper end is fixed to an ejector magnet 402 and the lower end of the ejector pin should be at least 2mm higher than the orifice of the cutting head when the ejector pin is in its uppermost, retracted or "home" position. A solenoid 401 surrounds the ejector magnetic 402. A "spring" magnet 410 having a central bore along which the ejector pin is free to move is disposed between the ejector magnet 408 and the tip portion 404. The spring magnet 410 is oriented so that it repels the ejector magnet 402 upwards so that the ejector pin is normally retracted. However, in use, after the cutting head has been lowered onto a sample and has cut a sample from the gel or other base, activation of the solenoid 410 forces the ejector magnet downwards which in turn forces the cutting pin downwards and ejects the cut sample held in the cutting head.

When power is removed from the solenoid, the ejector magnet once again is repelled upwards by the spring magnet 410.

Figure 2b shows an alternative arrangement in which a spring 403 is used to keep the ejector magnet 402 in the "up" position instead of a magnet. In that embodiment, the solenoid 401 of the cutting tool body 400 is activated which drives the ejector magnet 402 down onto a spring mechanism 403. This forces the ejector pin 405 through the cutting head orifice 406 ejecting the sample into a microtitre plate. When the solenoid is deactivated the ejector magnet 402 is forced back up into the solenoid body by the expansion of the spring 403.

The cutting tip 416 shown in Figure 2b is disposable and is generally conical, with a gentle taper and an annular cross-section. It pushes or snap-fits onto a conical protrusion 418 depending from the body 400.

The cutting tool should be of sufficient length so that the ejector pin is at least 2 mm higher than the orifice of the cutting head when the ejector magnet is in the home position

The ejector pin should protrude from the cutting tool orifice by at least 1mm when the ejector magnet is forced down by the activation of the solenoid.

It is also possible to image the gel independently of the cutting apparatus, identify the coordinates of the spots to be excised and then transform the coordinates into robot xy coordinates. The gel is scanned and then transferred from the scanner onto the cutting table. To determine the coordinates so that the robot now knows where to cut, the robot is taught four landmarks, preferably the points that are the furthest NE, SE, NW and SW on the gel. This will derive a function which we can then transform the image derived coordinates into robotic coordinates. The xy data file is then used by the robot software to excise the spots.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Dated this twentieth day of May 1998

MACQUARIE RESEARCH LTD

Patent Attorneys for the Applicant:

F.B. RICE & CO.

FIG 1

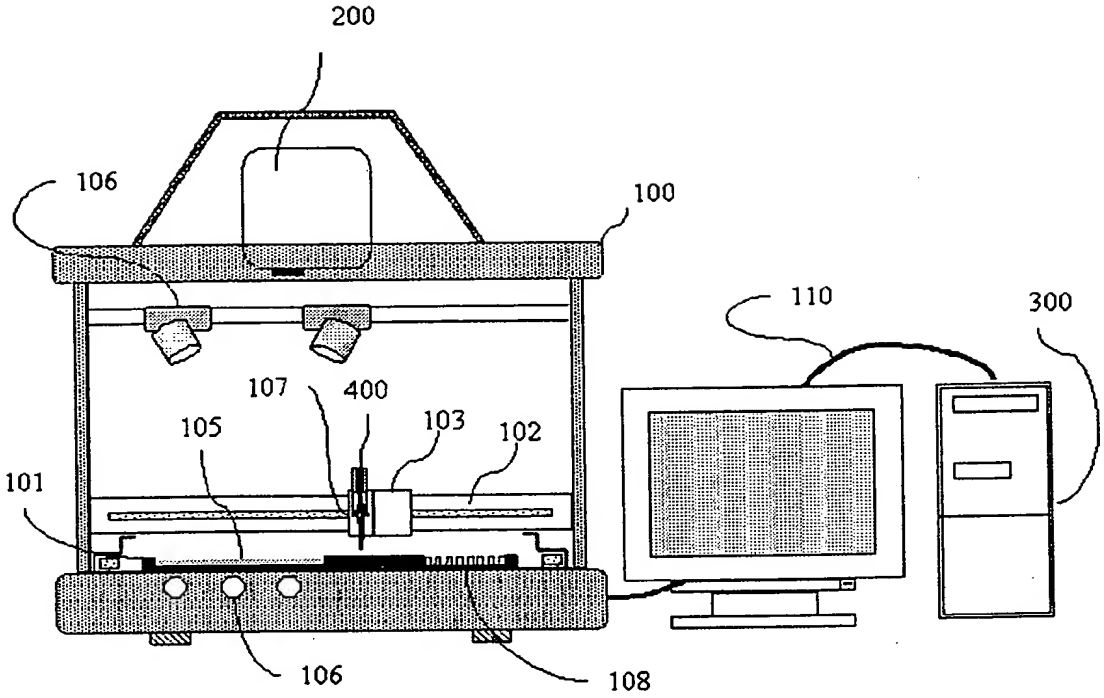

Fig. 2a

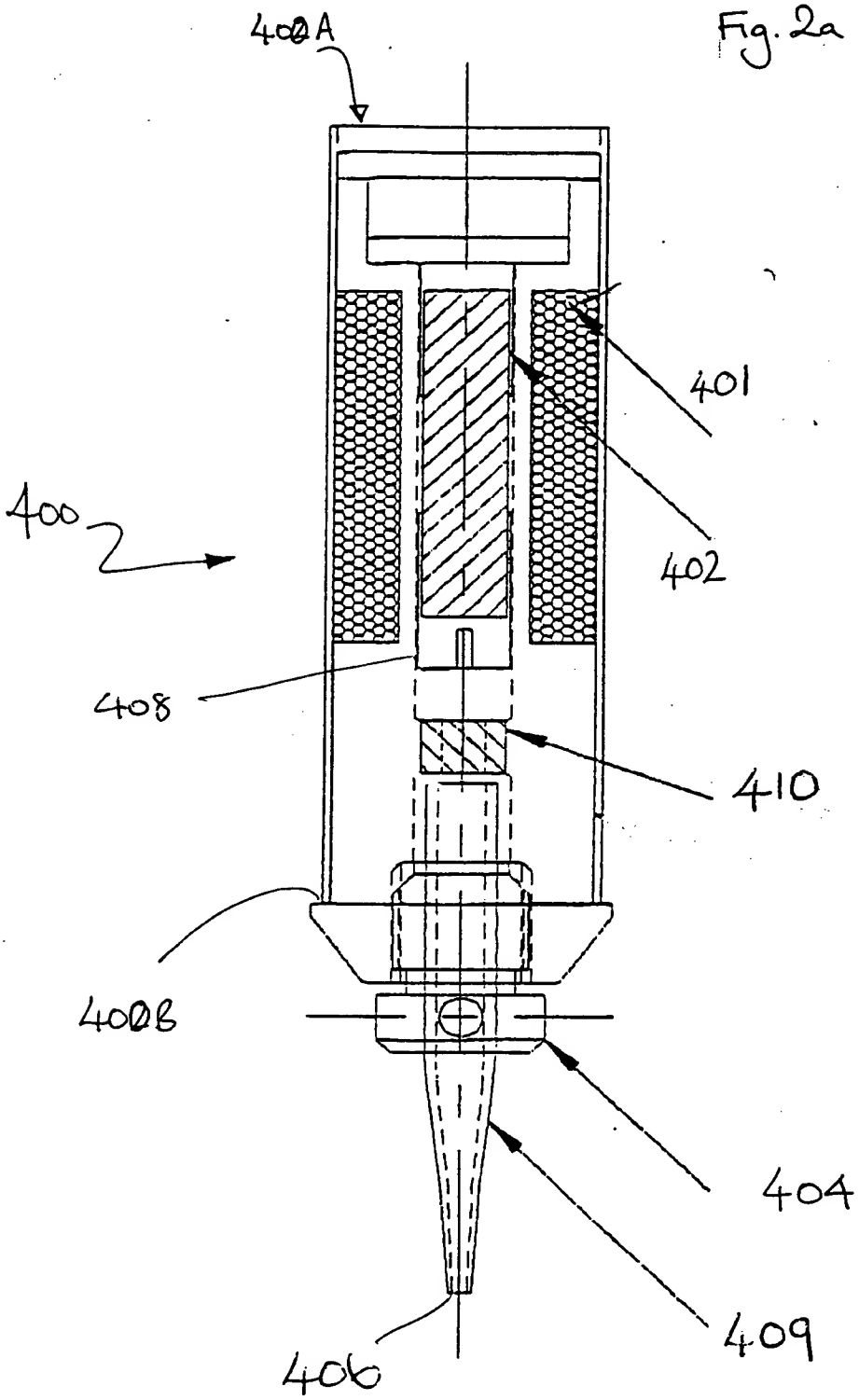

FIG 2b